NOTES ON THE CHROMATOLOGY

OF

ANTHEA CEREUS.

BY

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Notes on the Chromatology of Anthea cereus.

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With Plates XXXIX and XL.

The colouring matters of Anthea cereus were first examined by Sorby,[1] who found several present in this Actinia. Among others he found chlorofucin, the bands of which had been observed by Mr. Charles Horner, and the position of which led Mr. Horner to think that the supposed chlorophyll was different to that of land plants. Sorby had previously found chlorofucin in fresh-water algæ and subsequently in Fucus and other olive marine algæ; and in his paper on "Comparative Vegetable Chromatology" he gave directions for its separation from other pigments. Prof. Lankester, in the list of chlorophyll-containing animals in the English edition of Sachs's 'Botany,' includes Anthea cereus and puts "chlorofucin" after it, thus accepting Sorby's statements.

Among those animals which have been proved to contain symbiotic unicellular algæ Anthea is now, I believe, included;[2] and it becomes of interest to find out whether chlorofucin is due to the presence of these symbiotic algæ or whether it is a pigment belonging intrinsically to the animal; whether, also, the other colouring matters associated with the chlorofucin

[1] 'Proc. Roy. Soc.,' No. 146, vol. xxi, 1873, p. 454.

[2] Hertwig, O. and R., "Die Actinien," 'Jena'ische Zeitschrift. f. Naturwis.,' Bd. xiii, 1879, S. 495—500; and Geddes, 'Proc. Roy. Soc. Edin.,' vol. xi, 1881—1882.

belong to the animal or the algæ. I have already[1] proved this point almost completely, as I found that in Anthea cereus, in Bunodes Ballii, and Sagartia Bellis, "yellow cells," or symbiotic algæ, are present, that these animals all contain chlorofuein, all contain the same accompanying colouring matters, and that these colouring matters are evidently due to the "yellow cells" with which the tentacles are stuffed; for there is no essential difference in the spectra of the solutions of the tentacles in which the colouring matters are derived entirely from the "yellow cells" and those obtainable from other parts of the Actinia.

Moreover, I have also proved that in anemones possessing yellow cells there is more or less suppression of the respiratory proteids found in other Actiniæ.

But I had not repeated Sorby's experiments in which he applied Stokes's "fractional" method for the separation of the chlorofuein from the other colouring matters. In the present paper I have given the results of this examination, and, as will be seen, the statements of Sorby have been verified. This is of importance, as Krukenberg[2] has omitted to mention in the account of his experiments the results arrived at by Sorby, although, as I shall show, he had evidently chlorofuein before him in some of the solutions whose spectra he has mapped

In the paper referred to above[3] I have shown that the mixture of colouring matters obtained from the Actiniæ therein mentioned contain chlorofuein, and that the bands of this correspond to the chlorofuein bands in a similar solution of Fucus serratus. Sorby has figured in a diagram the bands of this pigment, but he does not give their wave-length measurements, and only figures the dominant bands of "blue" and "yellow chlorophyll" in the same diagram for the sake of comparison; consequently some confusion is caused when one endeavours to find out what

[1] 'Proc. Roy. Soc.,' No. 235, 1885, and 'Philos. Trans.,' 1885, Part II, 641, and seq.

[2] 'Vergleichend-Physiol. Studien.,' 1ste Reihe, 5te Abth., 1881, S. 38, and Ibid. 2te Reihe, 3tte Abth., 1882, S. 72.

[3] 'Philos. Trans.,' loc. cit.

Sorby means by "blue" and "yellow chlorophyll" and "chlorofucin," and the object of this paper is to clear up this confusion as regards chlorofucin more especially, also to prove that the chlorofucin and its accompanying pigments are due entirely to the "yellow cells."

It is necessary here to recall the experiment of Geddes[1] to mind. Geddes found that by exposing Anthea cereus to sunlight he got as much as 32—38 per cent. of oxygen, and he found starch and cellulose in the "yellow cells." He tries to reconcile this fact with the statement made by Krukenberg, who failed to get any evidence of the evolution of oxygen, by supposing that Krukenberg must have examined a different variety of Anthea; and he further observes: "Thus, then, the colouring matter of Anthea, described as chlorophyll by Lankester, has really been mainly derived from that of the endodermal algæ of the variety plumosa, which predominates at Naples, while the Anthea-green of Krukenberg must mainly consist of the green pigment of the ectoderm, since the Trieste variety evidently does not contain algæ in any great quantity. But since the Naples variety, contrary to the opinion of the brothers Hertwig,[2] does contain a certain amount of ordinary green pigment, and since the Trieste variety is tolerably sure to contain some algæ, Heider having indeed shown the presence of yellow cells in Sagartia, both spectroscopists have then been operating on a mixture of two wholly distinct pigments—one vegetable, the other animal—diatom-yellow and Anthea-green." In other words, Prof. Lankester's pigment would be "diatom-yellow" and Krukenberg's "Anthea-green." But I believe this theory will not account for the above-mentioned discrepancy, as I find a chlorophyll as well as a chlorofucin in extracts of the "yellow cells," and I shall endeavour to show that out of these yellow cells one extract may contain more of one coloured constituent, another more of another; and this result does not prove that one extract contains

[1] 'Nature,' 26th Jan., 1882, pp. 303—305, and 'Proc. Roy. Soc.,' Edin., vol. xi, 1881, 1882, p. 377—396.

[2] Loc. cit.

an intrinsic Anthea pigment and another an algal pigment, but simply this: that the colouring matters of the algæ are several, for we find at least one chlorophyll, one chlorofucin, and certain lipochromes, and perhaps other pigments, all of which belong to the "yellow cells."

The Physiological Proof is not wholly reliable. The evolution of oxygen in the presence of sunlight in the case of Anthea must to a large extent at least depend on the situation of the "yellow cells," for it is evident that if in a given species these are shut up in the tentacles the oxygen given off has not much opportunity of escaping out of them so as to make itself evident in a vessel containing the anemone, moreover a large amount of the oxygen will in such a position be largely absorbed by the tissues of the animal, and the same manner in which the cells are packed, as shown in the accompanying drawing, prevents the deeper lying cells from being acted upon by the rays of light. If on the other hand the ectoderm of the animal were studded with algæ there would be a considerable development of oxygen perceptible, and there is certainly such a variation in the distribution of the "yellow cells," for in some Actiniæ I have observed rows of algæ embedded in the ectoderm, while in others they may be mostly confined to the tentacles. This point should not be lost sight of, and may account for the discrepancy to a great extent.

The Morphological Proof that the "yellow cells" are parasitic algæ has been so well discussed that I need not here "treat of it," but I may observe that Krukenberg's idea as to their hepatic function must, so far as their microscopic character is concerned, be completely negatived. In no invertebrate liver or answering organ are such bodies found. On the contrary, the microscopic characters of liver chlorophyll or entero-chlorophyll at once separate it from that of the "yellow cells;" for, in the case of entero-chlorophyll, it is easy enough to see that it occurs mostly dissolved in oil, or in granules, or diffused through the protoplasm of the lining cells of the liver tubes.[1] The spectroscopic reasons for the same conclusion are considered below.

[1] 'Proc. Roy. Soc.,' No. 237, 1885, and 'Philos. Trans.,' 1886, Part I.

The Chemical Proof is no less convincing, as the presence of starch within the "yellow cells," and of a cellulose wall surrounding them, is easily proved, especially after, as Geddes has shown, the usual botanical precautions have been taken, namely, steeping the "yellow cells" in alcohol, then in caustic potash, and neutralising with acetic acid before applying the tests with iodine and with Schulze's fluid. The same tests applied to liver chlorophyll entirely fail.

It is not necessary here to describe the differences between the "yellow cells" and the chlorophyll corpuscles of Hydra and Spongilla, as Professor Lankester[1] has shown that the latter are not parasitic algæ, and I have[2] lately studied the chlorophyll corpuscles of Stentor polymorphus, Paramœcium, and Ophrydium, and compared their morphological characters with those of the "yellow cells," and have concluded that they too are not parasitic algæ, although in some corpuscles I have found traces of an amyloid substance, and the presence of a cellulose wall. Miss Jessie Sallitt[3] has also studied the morphology of the chlorophyll corpuscles of certain Infusorians, but she has not found starch. This, however, is of no importance, for there is no reason why starch should not appear in the protoplasmic contents of an animal chlorophyll corpuscle containing chlorophyll. Nor would the absence of starch within such cells justify us in concluding that it is not formed there, as it may be, and probably is, rapidly removed elsewhere as soon as it is formed (Lankester). Granting that starch is built up by the agency of chlorophyll from carbon dioxide and water, we may not always meet with it, for botanists teach that some "non-nitrogenous[4] organic substance is first formed in the chlorophyll corpuscle from carbon dioxide and water," which is "not starch, but a sub-

[1] "On the Chlorophyll Corpuscles and Amyloid deposits of Spongilla and Hydra," 'Quart. Journ. Mic. Sci.,' vol. xxii, N.S., p. 229.

[2] 'Proc. Birm. Philos. Soc.,' vol. v, Part 1, pp. 177—218.

[3] "On the Chlorophyll Corpuscles of some Infusoria," 'Quart. Journ. Mic. Sci.,' vol. xxiv, 1884.

[4] Vines, 'Lectures on the Physiology of Plants,' 1886, p. 115.

stance (possibly allied to formic aldehyde) which goes to construct proteid, by combining either with the nitrogen and sulphur absorbed in the form of salts from the soil, or with the nitrogenous residues of previous decompositions of proteid. The starch deposited in the corpuscle is, however, the first visible product of the constructive metabolism going on within it; for, unless protoplasm is being formed, no starch can be produced: it may be regarded as a temporary reserve material." The fact that such "reserve material," while, being of great service in a vegetable cell, and not being of much service in an animal cell, may lead to the metabolic process stopping short of its actual formation; for it appears to me that the principal use of chlorophyll in the animal cell may be to supply the animal with oxygen by the decomposition of the animal's waste carbon dioxide, and the formation of starch would be, to a great extent, a superfluous advantage. In that case the formation of starch would be more accidental than of actual necessity. This view of the function of animal chlorophyll is very much strengthened by the recent experiments of Regnard,[1] which, if confirmed, will tend to support the view that chlorophyll, even separated from the "chlorophyll corpuscles," is of use in the respiratory processes of animals. Whatever the rôle of the intrinsic chlorophyll of the animal may be, there can be little doubt as to that of the "yellow cells" of Anthea, which all proofs, morphological, physiological, chemical, and spectroscopic, point out as being distinct organisms, having an independent life from the animal, although, of course, benefitting by their position not only themselves but their host.

If sections of the tentacles are made after hardening in alcohol, it will be seen that the masses of yellow cells are packed in the tentacle at random as it were, and as the water is absorbed from them by the alcohol, radiating cracks appear in the mass of cells which are merely laid in apposition to each other, and not connected in any way as they would be if part of the animal's structure. I have endeavoured to show this in

[1] 'Compt. rend.,' CI, 1293—1295, and 'Journ. Chem. Soc.,' March, 1886, p. 254.

the accompanying drawing, an inspection of which alone will convince most people that these bodies are not "secreting cells" (Pl. XXXIX).

Results of Spectroscopic Examination.—The chlorophyll of Anthea differs from other chlorophylls in its remarkable instability towards caustic alkalies; this I have already described,[1] and the chlorofucin which accompanies it is also remarkably unstable. I propose first to describe the results of an examination of the solutions of these pigments, and then to compare them with plant chlorophyll.

In comparing my results with those of Krukenberg, the difficulty at once is encountered of attempting to find out what bands of his correspond with mine, as in all his early maps the Fraunhofer lines occupy the wrong position, and none of his measurements have been given in wave lengths. Still it is not difficult to see that the pigments met with by him do not differ from those here described, and if this be the case it is easy to say which are the bands of chlorofucin and which of chlorophyll, &c., in his drawings, as I shall show further on.

I now proceed to describe the results of an examination of the colouring matters of Anthea. All the specimens which I examined have been of a dull greenish colour and therefore are of the same colour as those examined by Sorby.

The tentacles were removed from several specimens and put into absolute alcohol after washing with water, this I may call solution (1). The other parts without the tentacles were cut up small, washed with water, and also put into absolute alcohol; this I may call solution (2). In both cases the absolute alcohol was left in contact with the parts for three days or longer in a dark place.

The colour of solution (1) was greenish yellow, and it had a red fluorescence and gave in a certain depth sp. 1, while in a shallow layer a band became detached in the violet end of the spectrum, which I have tried to represent by sp. 2; but it must be remembered that this band may not be exactly represented owing to the difficulty of seeing it, even by the help of

[1] 'Philos. Trans.,' loc. cit.

good daylight. The presenee of the second band (commencing from the red end) at once stamps this speetrum and distinguishes it from the usual ehlorophyll speetrum. These bands read approximately :

1st band	. . .	λ 674·5 to λ 653.
2nd „	. . .	λ 641 to λ 625.
3rd „	. . .	λ 595 to λ 575.

While the 4th band was guessed to be λ 467 to λ 443, but its edges were so ill-defined and it was so encroached upon by the general absorption of the violet end that this measurement may rcquire to be eorrected.

The colour of solution (2) was a deep orange and it had also a red fluoreseence, but not so well marked as that of the former solution. The spectrum is shown in sp. 3, and it is seen that while the same bands are present as those of sp. 1, yet the two first are of relatively different intensity of shading. This points to the obvious eonclusion that two eolouring matters are present, the one indieated by the band in red, the other by the seeond band (and the third, as will bc shown further on), there is more of the former present in the solution of the tentacles, of the latter in that of the other parts. The readings of these bands were the same as the last. In a thinner layer of this solution (2) there are two bands nearer the violet, that nearer the red being less shaded than the other. Sp. 4 is an attempt to show this, but it only approximately represents the position of these bands, of whieh one is coincident with the lipochrome band of sp. 2. This difference of spectrum explains the diffcrence of eolour of these solutions, for the latter solution probably eontains an additional yellow colouring matter probably derived from the animal, but it is not an additional green but a yellow.

Solution (1) (of the tentaeles) was now agitated in a separating funnel, after dilution with water, with earbon bisulphide, when the bisulphide fell to the bottom of a yellow-green eolour leaving the alcohol layer orange. This bisulphide solution on separating possessed a red fluorescence and gave sp. 5, and the following readings :

1st band . . . λ 685 to λ 656.
2nd „ . . . λ 647 to λ 612·5. (?)
3rd „ . . . λ 597 to λ 573.

Two other bands in the green were also seen which doubtless are obscured in the alcohol solution by the shading produced by the yellow constituents absorbing the violet end; and two others in the violet end were also visible, which, as far as I could judge, measured approximately from λ 479 to λ 460, and perhaps λ 453 to λ 436.

A second bisulphide extract of the same solution, after the first had been removed, was not nearly as green as the first bisulphide extract, and contained less of the constituents giving the bands in the red half of the spectrum, but showed a dark band in the blue which was coincident with the λ 479 to λ 460 band just mentioned. This spectrum is shown in sp. 6.[1] In every other respect the bands of sp. 5 and 6 agree.

A third bisulphide extract of the same solution was hardly coloured, and gave traces of the same bands. The alcohol solution, which had been thrice extracted with bisulphide, was of an orange colour and contained a good deal of bisulphide, and now this solution no longer showed the first band in red, but did show the second and third bands of the original spectrum; these latter are evidently the bands of Sorby's chlorofucin, as can be seen by a comparison with his diagram. They are shown in sp. 7; in a thinner layer there are other bands nearer the violet measuring approximately λ 511 to λ 488, and λ 477 to λ 457, as shown in sp. 8.

To see whether these two latter were the bands of Sorby's fucoxanthin, a couple of drops of ammonia were added and a little water; the bisulphide layer was now turbid and gave one broad band in green, the solution having an amber tint. (It is noticeable that in this and in a second similar bisulphide extract the dark bands in the blue and violet of sp. 6 and 8 were not present; but the blue colour with hydrochloric acid

[1] Possibly the band may not belong to a lipochrome, as I found a pigment in Anthea's tentacles soluble in glycerine, with a band in this part of the spectrum. See below.

described by Sorby was not produced, because this pigment was not fucoxanthin.) The chlorofucin bands of sp. 7 after the bisulphide had removed as much colouring matter as possible was (approximately) :

1st band . . . λ 644 to λ 627·5.
2nd „ . . . λ 595 to λ 581.

There were, however, other bands present—one in blue green and one in violet. This solution was a deep yellow colour, due, probably, partly to the incomplete separation of the yellow pigment giving the bands in the violet end.

On evaporation of this solution some brown-yellow flocks separated out; the residue was extracted with absolute alcohol. In this it formed a fine deep yellow solution, and gave sp. 7 again, the bands reading :

1st band . . . λ 641 to λ 627·5.
2nd „ . . . λ 595 to λ 579.

What the alcohol left undissolved was nearly all taken up by distilled water, forming a yellow solution, giving some shading at the blue end of green and absorbing the violet end. Hydrochloric acid did not seem to affect this solution.

According to Sorby,[1] the alcohol solution of chlorofucin is changed by hydrochloric acid, and on adding this reagent to its alcohol solution a new spectrum appears, namely, sp. 9, whose bands read approximately :

1st band . . . λ 607·5 to λ 597.
2nd „ . . . λ 585 to λ 573.

Hence there is no doubt that this colouring matter was chlorofucin.

Solution (2) (i. e. the alcohol solution of the parts of Anthea without tentacles) also contained chlorofucin, as I proved, by a similar method. It was stated above that the solution (1), from which the bisulphide had removed as much of the colouring matter as it could take up, had been treated with ammonia (two drops) and agitated afresh with bisulphide, and that this

[1] Loc. cit., p. 455.

bisulphide gave a band in the green; such a solution was evaporated to dryness, and the residue tested by the lipochrome tests. With iodine and iodide of potassium it remained unchanged, with nitric acid it gave a transient blue, with sulphuric acid a green and blue; hence it is a lipochrome.

A second absolute alcohol extract having been made from the tentacles only was of a greenish-yellow colour with a red fluorescence, and gave the following bands:

1st band . . . λ 681·5 to λ 650 (dark part = λ 678 to λ 653).
2nd „ . . . λ 636 to λ 601.
3rd „ . . . λ 593 to λ 573. (See Sp. 10.)

In a thin layer there may have been some shading between green and blue, and perhaps some in violet. To this solution water was added, and it was then agitated with carbon bisulphide. On separating the latter it was greenish, leaving the alcohol yellow, and in both these solutions certain bands in the violet could now be seen, which were not visible in the original alcohol solution (before separation). I have tried to show all the bands in sp. 11 and 12. These read as follows:

1st band . . . λ 686 to λ 656.
2nd „ . . . λ 644 to λ 612·5.
3rd „ . . . λ 599 to λ 575.
4th „ . . . (a shading before the next).
5th „ . . . λ 523 to λ 496.
6th „ . . . λ 479 to λ 460.
7th „ . . . λ 453 to λ 436.

Now, on comparing these bands with the bisulphide extract from the first alcohol extract of the tentacles, they are found to be practically almost the same. The yellow alcohol solution from which the bisulphide had removed the pigment giving these bands, no longer contained any chlorofucin, and showed hardly a trace of a band in red in a deep layer. It did show the shadow of a band in green, and perhaps another in the violet.

If we compare a second absolute alcohol extract of the parts of Anthea free from tentacles with the second absolute extract of the tentacles, we find that no great difference exists between

them. Such a solution was greenish yellow; it possessed a fine red fluoreseence, and its bands read as follows:

1st band	. . .	λ 681·5 to λ 650.
2nd „	. . .	λ 638·5 to λ 605.
3rd „	. . .	λ 593 to λ 573.

This solution, diluted with water, was agitated with bisulphide; the latter fell down of a yellow colour, and possessed a red fluoreseence, and gave a speetrum the same as sp. 11, as will be seen by the following readings:

1st band	. . .	λ 685 to λ 656.
2nd „	. . .	λ 644 to λ 612·5 (?).
3rd „	. . .	λ 599 to λ 577.
4th „	. . .	?
5th „	. . .	λ 523 to λ 499.
6th „	. . .	λ 479 to λ 460.
7th „	. . .	λ 453 to λ 436.

The alcohol solution from which this solution had been removed was of a yellow colour, and gave a feeble band at the end of green, and one in violet; on evaporation it left a dirty yellow residue, which gave no reaction with iodine and iodide of potassium, was slightly green with nitric acid, and brownish with sulphuric acid.

A third absolute alcohol extract of the tentaeles only, which had a green colour and a red fluoreseence, gave sp. 13. The only difference between this and sp. 11 is in the shading of the second band, as in 13 it is one single band, whereas in 11 there is a distinct narrow part over the broad, less shaded one. The wave length readings, too, show a close agreement; thus the bands read:

1st band	. . .	λ 678 to λ 647.
2nd „	. . .	λ 633 to λ 601.
3rd „	. . .	λ 591 to λ 571.

There was also a band at the blue end of green, and perhaps one in violet.

A third extract of the parts of Anthea without the tentacles contained the same colouring matters. It was of a

greenish colour with a blood-red fluorescence, and gave the following bands :

1st band	. . .	λ 678 to λ 647.
2nd ,,	. . .	λ 633 to λ 601.
3rd ,,	. . .	λ 591 to λ 573.

There was also a band at the blue end of green, and perhaps one in violet. Hence, then, the third extract of the tentacles and the third extract of the parts without the tentacles contain the same colouring matters. Now, the colouring matter in these latter extracts, which gives the bands in the red end of the spectrum, is almost, if not altogether, free from chlorofucin ; at least the second and third bands of the spectrum give different measurements. So far as I can judge this, then, must be the pigment which Krukenberg would have belonging to Anthea, which he calls "Antheen," although I cannot prove this assertion, owing to the way his spectra are represented. Krukenberg says that the corresponding pigment withstands saponification, but in this case, as I have previously shown,[1] the addition of a little caustic soda alters the spectrum, as shown in sp. 14.[2]

A fourth absolute alcohol extract of the tentacles gave the same spectrum, the bands reading :

1st band	. . .	λ 678 to λ 650.
2nd ,,	. . .	λ 633 to λ 601.
3rd ,,	. . .	λ 591 to λ 573. Others uncertain.

A fourth similar extract of parts without tentacles read :

1st band	. . .	λ 678 to λ 650.
2nd ,,	. . .	λ 633 to λ 601.
3rd ,,	. . .	λ 591 to λ 573. Others uncertain.

And a fifth and sixth alcohol extract of the tentacles, and a fifth and sixth of the parts without them, gave the same readings. Hence we have precisely the same colouring matters present in the tentacles as in the rest of the animal, and as those in the tentacles are due to

[1] 'Philos. Trans.,' Part II, 1885.

[2] NaHO causes precipitation, but after filtering, the solution is green, and gives sp. 14.

"yellow cells," it is fair to conclude that those of the latter are also due to "yellow cells," and do not belong intrinsically to the animal, which is the point to be proved.

So far, then, there are several pigments present, three at least; one represented by a chlorophyll-like spectrum, characterised by the dominant band in red, and by others when the chlorofucin is separated out, chlorofucin represented by sp. 7, and a lipochrome, or lipochromes, represented by bands in the violet half of the spectrum. The chlorophyll is, however, more decomposable by caustic alkalies than is that of land plants; and it is now necessary to see whether it agrees with Sorby's "blue" or "yellow chlorophyll." Sorby's yellow chlorophyll is found in Ulva latissima.[1]

In the following experiments it must be remembered that a relatively large quantity of chlorophyll was present, and that consequently the dominant chlorophyll band was much broader than that in any of the above spectra. I did not boil the Ulva, as Sorby directs, but merely extracted it in the cold with absolute alcohol for three days in the dark. The resulting solution was a fine sap green colour with a blood-red fluorescence, and gave in a suitable depth the following measurements:

1st band	. . .	λ 681·5 to λ 641.
2nd „	. . .	λ 625 to λ 599.
3rd „	. . .	λ 591 to λ 566.
4th „	. . .	λ 549 to λ 532.

There were also two other bands, one in blue green and one in violet. But in order to compare this solution with the chlorophyll constituent of Anthea's colouring matters, it is necessary to agitate with bisulphide of carbon and examine the latter solution. Putting now the measurements of the bands of this solution, and those of the corresponding extract of Anthea side by side, we get:

[1] Sorby, loc. cit., p. 453. Besides the lipochromes there is in Anthea's tentacles a pigment soluble in glycerine which gives bands in the violet, which I have described in my paper on the "Chromatology of the Actiniæ," loc. cit.

	Ulva.	Anthea.
1st band . .	λ 688·5 to λ 659 and shaded to λ 641.	λ 686 to λ 656.
2nd „ . .	λ 633 to λ 612·5.	λ 644 to λ 612·5.
3rd „ . .	λ 597 to λ 571.	λ 599 to λ 575.
4th „ . .	Uncertain.	Uncertain.
5th „ . .	λ 520 to λ 496.	λ 523 to λ 496.
6th „ . .	λ 485 to λ 466.	λ 479 to λ 460.
7th „ . .	λ 455 to λ 438.	λ 453 to λ 436.

The discrepancy in the measurements of the 2nd band of Ulva and Anthea may be due to a trace of chlorofucin in the bisulphide extract of the latter. The bands in the violet may be neglected as they belong to the lipochrome constituents. Hence it would appear that the chlorophyll constituent in Anthea is closely related to, if not identical with, "yellow chlorophyll." A comparison of sp. 11 and 12 with sp. 15 and 16, teaches that there is a remarkable resemblance between the pigments of Ulva and Anthea not only as regards the chlorophyll bands in the red half of the spectrum, but also as regards the lipochrome bands in the violet half.

I have not here considered the identity of the chlorofucin of Anthea with that found in Fucus serratus, as I have done so already, but I may add that I have lately separated chlorofucin by Sorby's method from other olive algæ, such as Fucus nodosus, and Laminaria digitata, and find that the bands are identical with those of the chlorofucin of Anthea.

We may now conclude that the pigments of Anthea are the pigments of certain marine algæ, and are therefore the pigments of the "yellow cells," which are known to be unicellular algæ.

With regard now to Krukenberg's results; taking his first paper[1] and examining the plate attached to it, we find that in the first spectrum he figures the double band in the red, the second of which I have shown to belong to chlorofucin; the second spectrum represents the effect of NaHO on "Anthea-green," but it really represents the effect of NaHO on

[1] 'Vergleichend. physiol. Studien.,' 1te Reihe, 5te Abth., 1881.

chlorofucin and yellow chlorophyll mixed; the third spectrum also evidently represents the bands of both pigments, and the fourth shows a chlorofucin spectrum. In his second paper, which deals with Anthea viridis var. plumosa, the plate (Tafel iv)[1] shows clearly enough the presence of the same colouring matters, thus in sp. 2 the bands of chlorofucin are visible; in sp. 3 the chlorophyll constituent is present, perhaps mixed with the former, and so in all the others the presence of the same pigment with the lipochrome or lipochromes can be detected. Hence there is no essential difference between the pigments of Anthea plumosa and Anthea cereus, and doubtless, in the former, they are all due to the "yellow cells" also. Krukenberg seems to lean to the opinion that these colouring matters are allied to what he calls "hepatochromates," but such a theory is easily controverted, because (1) enterochlophyll is not nearly so easily decomposed as are the pigments of Anthea, and (2) the "fractional" method distinguishes them at once: in the case of enterochlorophyll, the bisulphide takes up more of the lipochrome, the alcohol retaining some chlorophyll. There are other reasons[2] which I have given elsewhere, and the morphological differences are so well marked that it is no longer possible to maintain such a view.

The present paper contains only a preliminary account of the subject, which I hope to study more thoroughly again.

I have gone over most of Sorby's experiments during the last ten years, and I am more and more convinced of the soundness of his deductions and the accuracy of his experiments; but owing to his diagrams not giving all the bands, and his bands not having all been described in wave-lengths, one has great difficulty sometimes in following the descriptions. This remark more especially applies to the chlorophylls, as I am unable yet to distinguish "blue" from "yellow chlorophyll," and I think one is safe in concluding with recent observers that the green constituent of chlorophyll gives four bands in the red half of the spectrum, and the yellow those in the violet half;

[1] Ibid., 2te Reihe, 3tte Abth., 1882.

[2] E. g., the spectra are totally different.

and hence I am inclined to think that the band represented in Sorby's diagram in the case of "blue" and "yellow chlorophyll" and chlorofucin occurring in the violet end, really belongs to the yellow constituent, which has been incompletely separated by the "fractional" method.

EXPLANATION OF PLATES XXXIX and XL.

Illustrating Dr. C. A. Mac Munn's "Notes on the Chromatology of Anthea cereus."

PLATE XXXIX.

Transverse section of a tentacle of Anthea cereus which had been kept in alcohol for some time; it was stained with picrocarmine, and mounted in Canada balsam. The relationship of the "yellow cells" to the endodermal lining of the tentacle is well shown. The specimen is supposed to be magnified 80 diameters, and was drawn by means of Swift's erecting camera lucida, the paper being fourteen inches distant from the eye-piece.

The radiating cracks in the interior of the mass of "yellow cells" are clearly shown, as well as the absence of any connecting structure.

PLATE XL.

Spectra of the colouring matters of Anthea cereus, &c.

Sp. 1.—Absolute alcohol extract of tentacles of Anthea, deep layer.

Sp. 2.—The same shallow layer.

Sp. 3.—Absolute alcohol extract of other parts without tentacles.

Sp. 4.—The same shallow layer.

Sp. 5.—The alcohol solution of tentacles was diluted with water and agitated with bisulphide of carbon, which gave this spectrum.

Sp. 6.—Second bisulphide extract from the alcohol solution; note the dark band in violet end, which does not belong to the pigment giving bands in the red end.

Sp. 7.—The original alcohol solution after having been three times extracted with bisulphide; these are the bands of Sorby's chlorofucin.

Sp. 8.—The same shallow depth.

Sp. 9.—Action of hydrochloric acid on the same solution, showing changed chlorofucin as Sorby describes it.

Sp. 10.—Second absolute alcohol extract of the tentacles; this shows mainly the chlorophyll bands, possibly the narrow band belongs to the chlorofucin ?

Sp 11.—This was diluted with water and agitated with bisulphide of carbon which on separating gave this spectrum.

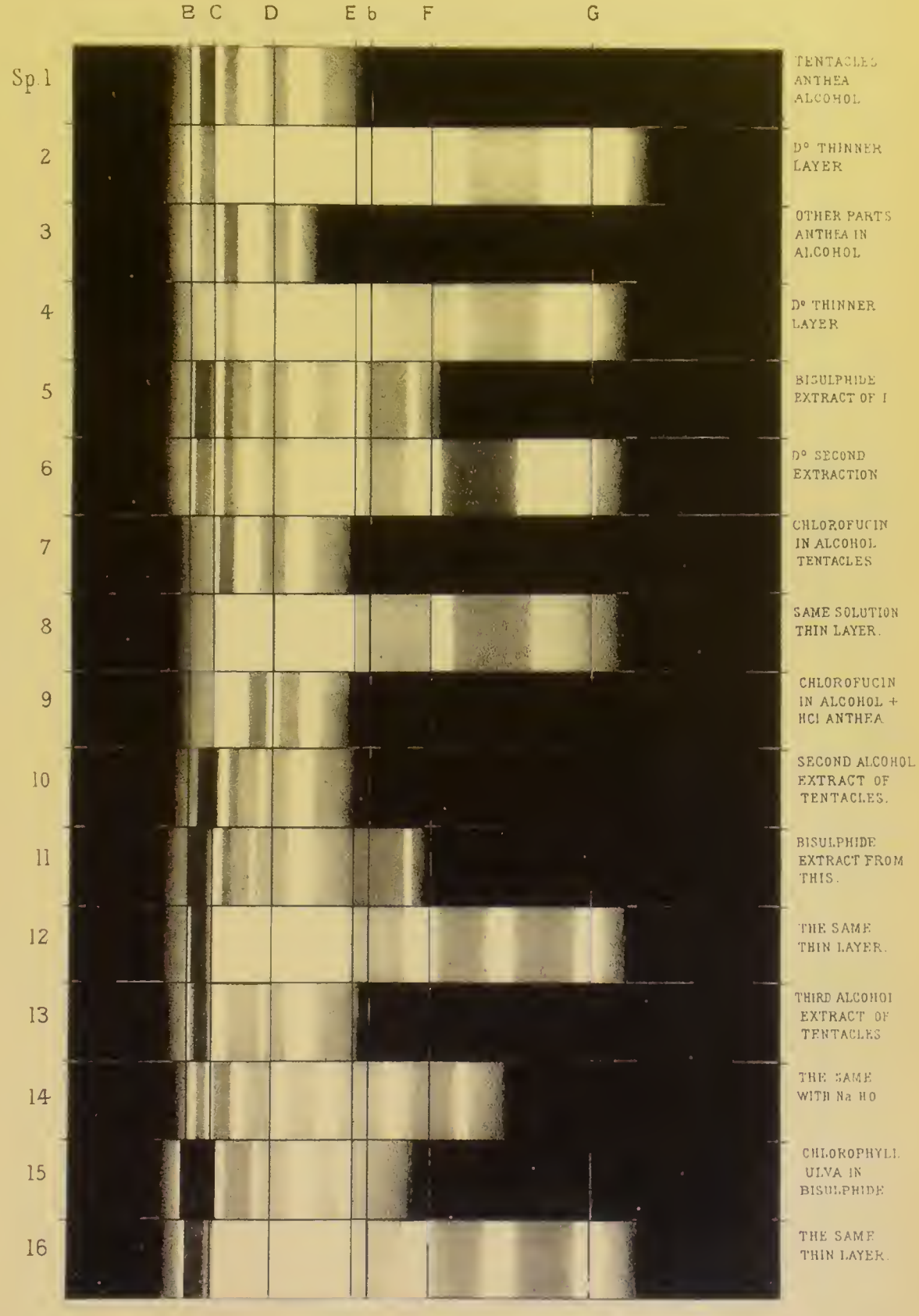
Sp. 12.—The same shallow depth.

Sp. 13.—Third absolute alcohol extract of tentacles (the second and third extract of the parts without the tentacles gave the same bands as the second and third extract of the tentacles, therefore they have not been mapped). Note in 13, the narrow (second) band is gone, showing that now the chlorophyll constituent is probably alone present.

Sp. 14.—Action of caustic soda on the last solution.

Sp. 15.—For comparison with the chlorophyll bands of Anthea. An absolute alcohol extract of a green Ulva, was diluted with water and agitated with carbon bisulphide, which after separation gave this spectrum; this solution appears to contain Sorby's "yellow chlorophyll." Compare with 5, 11, and 13.

Sp. 16.—Thin layer of the same. Compare with 12, and it will be seen that probably a similar lipochrome is present in Anthea and in Ulva.



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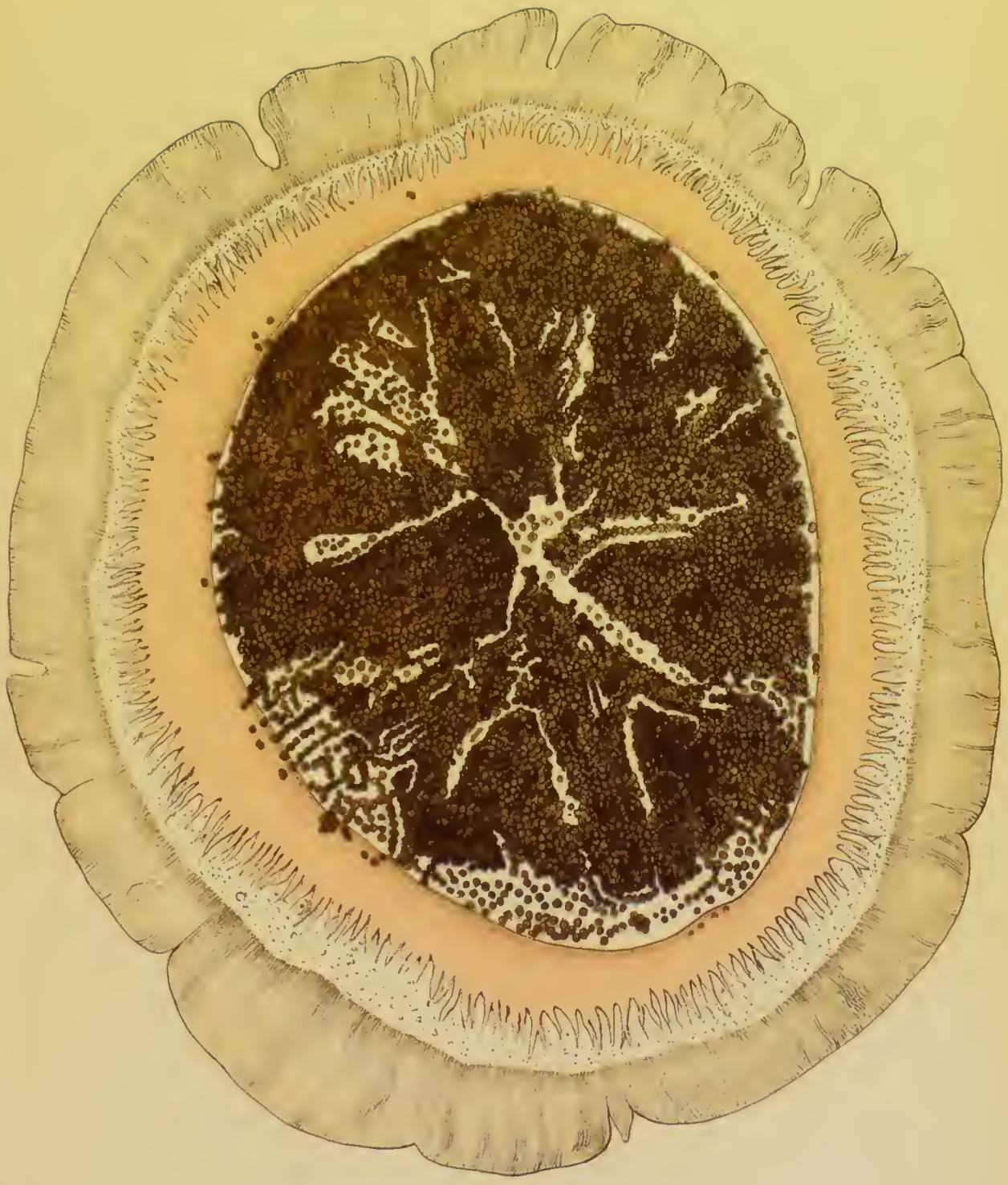

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